terminal region. This helix has been thought to help to coordinate conformational changes by linking discontinuous TM helices between the antiporters. Here, we introduced alanine-substitution mutation into eight residues; MrpA-D77, MrpA-R262, MrpA-G311, MrpA-F357, MrpD-D75, MrpD-D258, MrpD-G309, MrpD-F341 residues conserved in all MrpA, MrpD, NuoLMN to probe their roles in function and structure impact of MrpA and MrpD. Only mutations in MrpD, in residues it shares with MrpA, showed effects in function or complex formation, while no significant phenotypes were detectable in the mutants at corresponding residues in MrpA. We previously reported that MrpD is essential for expression of other Mrp subunits in the cytoplasmic membrane [1]. These data suggest that MrpD occupies primary position in Mrp complex.

Reference

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S4.P15

Purification and characterization of mitochondrial complex I from different tissues of *Rattus norvegicus*

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Mitochondrial complex I (NADH:ubiquinone oxidoreductase) is an entry point of electrons into the respiratory chain. It oxidizes NADH, reduces quinone, and couples the redox process to proton translocation across the inner mitochondrial membrane. Numerous diseases have been reported to arise from defects in complex I, ranging from muscular to neurodegenerative diseases [1]. Complex I has a unique L-shaped structure, containing 14 conserved 'core' subunits that have been identified in bacteria to eukaryotes [2]. Complex I from bovine heart has been used as a mammalian enzyme model for complex I studies, and 30 additional 'supernumerary' subunits have been identified in it [2]. However, it is not known how the subunit compositions of complex I from different mammalian tissues may vary. Rat (*Rattus norvegicus*) is a popular in vivo model for studying mitochondrial dysfunction and other diseases, providing a model for comparison of various tissues to study complex I using minimum materials. Here, tissues were obtained from the rat skeletal muscle, heart, kidney, liver and brain and used to prepare mitochondria and purify complex I. As there is no published protocol for obtaining highly-pure catalytically-active complex I from rat tissues, the standard bovine heart complex I isolation protocol was used initially [3], and modified to suit the small scale purification. A microscale purification system consisting of three chromatographic steps was applied in order to obtain the best resolution, recovery and purity from small amounts of material (down to 0.5 g tissue). The catalytic activities were assessed and the polypeptide composition of each complex I from each tissue was identified and compared by mass spectrometry.

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S4.P16

Site-specific chemical modification of mitochondrial NADH–ubiquinone oxidoreductase (complex I) through ligand-directed tosylate chemistry

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The reaction mechanism of mitochondrial NADH–quinone oxidoreductase (complex I) is thought to involve dynamic conformational changes in the membrane domain including the quinone catalytic site. The site-specific chemical modification of complex I by various functional probes such as fluorophores, without affecting the enzyme activity, would allow single-molecule analyses of putative conformational changes of the enzyme. To address this challenge, we tried site-specific alkynylation of complex I in bovine heart submitochondrial particles (SMP) through ligand-directed tosylate (LDT) chemistry using a synthetic high affinity ligand (acetogenin ligand 1), which has an alkynylated tosylate in the tail moiety. The terminal alkyne in ligand 1 was chosen as the tag to be incorporated into the enzyme since this functional group can work as a "footing" for subsequent diverse chemical modifications via click chemistry (i.e. azide-alkyne [3 + 2] cycloaddition in water). We carried out the alkynylation of complex I via LDT chemistry by incubating bovine SMP with ligand 1 for 24 h at 35 °C. To identify the alkynylated position by ligand 1, fluorescent TAMRA and/or biotin were covalently attached to the incorporated alkyne by click chemistry after the solubilization of complex I. Careful proteomic analyses showed that the alkynylation occurred at Asp160 in the 49 kDa subunit, which may be located in the putative quinone binding cavity. The alkynylation was completely suppressed by an excess amount of other inhibitors binding to the quinone cavity such as bullatacin and quinazoline. While the reaction yield of the alkynylation step was estimated to be ~50%, the alkynylation unfortunately resulted in the almost complete loss of the enzyme activity. Nevertheless, the results of our work demonstrate that complex I can be site-specifically alkynylated through LDT chemistry, providing a positive clue to diverse chemical modifications of the enzyme in combination with click chemistry.

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S4.P17

Role of protein-associated quinones Q-Nf and Q-Ns in complex I energy coupling

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Ohnishi and coworkers characterized complex I redox centers, identified two distinct protein-associated ubisemiquinone, SQ_{Nf} and SQ_{Ns},